INFECTION AND IMMUNITY, Mar. 1987, p. 698–703
0019-9567/87/030698-06$02.00/0

Vol. 55, No. 3

Metabolism of Glycosylsucrose by Oral Microorganisms and Its Hydrolysis by *Streptococcus salivarius* Fructosyltransferase

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Received 5 September 1986/Accepted 21 November 1986

Resting-cell suspensions of oral microorganisms grown in sucrose were studied for the production of acid from glucosylsucrose and maltosylsucrose. Most oral microorganisms fermented these sugars to only a limited extent. *Streptococcus salivarius*, however, metabolized glucosylsucrose as well as sucrose. We therefore looked for a specific enzyme in *S. salivarius* which was capable of hydrolyzing glucosylsucrose. Fructosyltransferase and invertase were purified from *S. salivarius* 13419, and the substrate specificities and hydrolytic activities of these enzymes were determined. Purified fructosyltransferase catalyzed fructan synthesis from glucosylsucrose or maltosylsucrose, whereas purified invertase barely hydrolyzed these sugars. These results suggest that the high fermentative efficiency of glycosylsucrose by *S. salivarius* is due to the hydrolysis of these sugars by fructosyltransferase, but not by invertase. The partially purified fructosyltransferases of *Actinomyces viscosus* NY1 and *Streptococcus mutans* NCIB 11723 catalyzed fructan synthesis from glucosylsucrose or maltosylsucrose. The fructosyltransferases of these oral microorganisms are also responsible for the hydrolysis of glycosylsucrose.

Glycosylsucrose is a general term for a maltooligosaccharide with a reducing end terminating in sucrose. A mixture of glycosylsucrose is obtained when starch and sucrose are incubated in the presence of a cyclodextrin glucosyltransferase (11). Glucosylsucrose and maltosylsucrose are the principal constituents of this glycosylsucrose mixture. The glycosylsucrose mixture inhibits insoluble glucan synthesis by *Streptococcus mutans*, and substitution of this sugar mixture for sucrose in the rat diet reduces the incidence of experimental caries induced by *S. mutans* (10). A cell suspension of *S. mutans* produced only a small amount of acid from glucosylsucrose and maltosylsucrose (20). Additionally, Yamada et al. (19) reported the limited acidogenicity of glucosylsucrose and maltosylsucrose in dental plaque in situ. These results indicate that the glycosylsucrose mixture could be a sugar substitute with low cariogenic potential. However, little is known concerning the degradation of glycosylsucrose by other predominant acidogenic microorganisms in human dental plaque. The present study, therefore, reports the acid production from glucosylsucrose and maltosylsucrose by various oral streptococci, actinomyces, and lactobacilli. In addition, we identified a specific enzyme present in *Streptococcus salivarius* that hydrolyzes glucosylsucrose as well as sucrose.

MATERIALS AND METHODS

Microorganisms and cultivation. *Streptococcus sanguis* LPH, NCTC 10904 (strain 804), and ATCC 10556, *S. salivarius* 13419, ATCC 9759, and *S. mutans* NCIB 11723 (strain JC2) were kindly supplied by J. Carlsson, Umeå, Sweden. *Streptococcus mitior* 115B2 and NCTC 10712 and *Streptococcus milleri* NCTC 10708 and 111C4 were kindly supplied by S. Edwardsson, Malmö, Sweden. *Lactobacillus casei* IAM 1045, *Lactobacillus acidophilus* IAM 1084, and *Lactobacillus plantarum* IAM 1041 were gifts from the Institute of Applied Microbiology, Tokyo University, Tokyo, Japan. *Actinomyces viscosus* NY1, WVU626, and Frank 1, *Actinomyces israelii* WVU307, and *Actinomyces naeslundii* NCTC 12104 were kindly supplied by J. S. van der Hoeven, Nijmegen, the Netherlands. These strains were stored in a lyophilized state. During the investigation these strains were subcultured monthly on blood agar plates and kept at 4°C.

The medium for the continuous culture of streptococci contained (in 1 liter of 100 mM potassium phosphate buffer [pH 7.0]): sucrose, 2.7 g; L-cysteine hydrochloride, 0.1 g; sodium L-glutamate, 1.0 g; NH_4HCO_3, 2.0 g; $MgSO_4 \cdot 7H_2O$, 0.02 g; NaCl, 0.01 g; $MnSO_4 \cdot 4H_2O$, 0.01 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; yeast extract, 2 g. For growth of batch cultures of streptococci, the same medium was used except that glucose (10 g) was used instead of sucrose. The medium for the continuous culture of actinomyces contained (in 1 liter of 100 mM potassium phosphate buffer [pH 7.0]): sucrose, 2.7 g; Trypticase peptone (BBL Microbiology Systems [Div. Becton Dickinson and Co.], Cockeysville, Md.), 10 g; yeast extract, 2 g; L-cysteine hydrochloride, 0.1 g; NH_4HCO_3, 2.0 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; NaCl, 0.01 g; $MnSO_4 \cdot 4H_2O$, 0.01 g; $FeSO_4 \cdot 7H_2O$, 0.01 g.

A 10-ml sample of a 12-h-old culture of streptococci or actinomyces in brain heart infusion broth was transferred to 400 ml of the continuous culture medium described above. The organisms were grown under sucrose limitation at a dilution rate (D) of 0.1 h^{-1} under an atmosphere of nitrogen at 35°C and pH 7.0. The culture was constantly stirred at 300 rpm. Steady state was attained by 3 days as judged by turbidity at 660 nm. Lactobacilli were cultured at 35°C for 24 h in 200-ml batches of MRS medium (4) supplemented with 1% (wt/vol) sucrose.

Acid production by resting cells. Cells were harvested by centrifugation (8,000 × g for 10 min at 4°C), washed twice with 50 mM potassium phosphate buffer (pH 6.8), and then were suspended in the same buffer. The reaction mixture contained (in 2 ml): 100 mM potassium phosphate buffer (pH 6.8), 5 mM $MgCl_2$, 10 mM sugar, and cell suspension (25 mg

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[dry weight]). Experiments dealing with acid production were performed anaerobically (20 min at 35°C) in an anaerobic glove box (type NHC [21]) under an atmosphere of 80% N_2, 10% CO_2, and 10% H_2. Fermentation was stopped by the addition of 0.2 ml of 25% (wt/vol) metaphosphoric acid, and cells were removed by centrifugation (8,000 × *g* for 10 min at 4°C). The clarified supernatant fluid was removed and analyzed for fermentation products. The lactate concentration was determined with L-lactate dehydrogenase from rabbit muscle (9). Other volatile acids were analyzed by gas chromatography as described previously (18).

Reducing sugar production from oligosaccharides by cell extracts of streptococci and actinomyces. Hydrolytic activity was estimated by measuring the reducing sugar formed from the nonreducing substrates following the procedure described previously (20).

Studies with decryptified *S. salivarius* cells. *S. salivarius* 13419, ATCC 9759, and ATCC 8614 were grown at 35°C overnight, harvested, and washed twice with 40 mM potassium phosphate buffer (pH 7.0) containing 5 mM $MgCl_2$. Cells were suspended in the same buffer and treated with 0.1 volume of toluene at 4°C for 90 s with vigorous agitation. The decryptified cells (5 mg [wet weight]) were washed three times with the same buffer. They were then suspended in 0.1 M potassium phosphate buffer (pH 7.0) containing 125 mM sugar (sucrose, raffinose, glucosylsucrose, or maltosylsucrose) and incubated at 37°C for 2 h. The reaction was terminated by heating the suspension in a boiling water bath for 10 min. The suspension was clarified by centrifugation (10,000 × *g* for 30 min at 4°C), and the supernatant fluid was removed and analyzed for reducing sugar and fructan.

Purification of fructosyltransferase from *S. salivarius* 13419. *S. salivarius* 13419 was grown overnight at 35°C. The cells were harvested and washed twice in 40 mM potassium phosphate buffer (pH 7.0). The location of fructosyltransferase in this bacteria was examined by the same method as that described by Garszczynski et al. (8). Fructosyltransferase was extracted from the cells with sodium chloride as described by Whitaker and Edwards (17). The solubilized enzyme was centrifuged at 17,500 × *g* for 30 min at 4°C, and the supernatant was dialyzed against 40 mM potassium phosphate buffer (pH 7.0) for 24 h. Solid ammonium sulfate was then added (with constant stirring) to 50% saturation. The resultant precipitate was collected by centrifugation (10,000 × *g* for 30 min at 4°C) and suspended in 40 mM potassium phosphate buffer (pH 7.0) followed by dialysis for 24 h against the same buffer. The enzyme preparation was applied to a Bio-Gel (Bio-Rad Laboratories, Richmond, Calif.) A-15 column (1.5 by 25 cm) equilibrated with 40 mM potassium phosphate buffer (pH 7.0), and the column was eluted with the same buffer. Fractions containing fructosyltransferase activity were pooled, concentrated, and then subjected to fast protein liquid chromatography (FPLC) on a MonoQ HR 10/10 column (10 by 10 mm; Pharmacia, Uppsala, Sweden). The column was run at a flow rate of 2 ml/min. The column was equilibrated in solution A (40 mM Tris hydrochloride buffer, pH 7.0), and a linear gradient of 25 fractions from 0 to 100% solution B (100 mM NaCl) was used for elution. Fractions containing fructosyltransferase activity were pooled, concentrated, and then subjected to FPLC on a Superose 12 column (10 by 300 mm; Pharmacia). The column was eluted with 40 mM potassium phosphate buffer (pH 7.0) at a flow rate of 0.5 ml/min. Fractions with fructosyltransferase activity were collected.

The molecular weight of the enzyme was estimated by FPLC on a Superose 12 column. The standard proteins used for calibration were ferritin (molecular weight, 540,000), catalase (240,000), aldolase (158,000), bovine serum albumin (68,000), and chymotrypsinogen (25,000).

Purification of invertase from *S. salivarius* 13419. *S. salivarius* 13419 was harvested at the early stationary growth phase, and the cells were washed twice with 40 mM potassium phosphate buffer (pH 7.0). After resuspension of the cells in the same buffer, the cells were disrupted by sonic oscillation (200 W, 2 A; model 200M sonifier; Kubota, Tokyo, Japan) for 15 min at 0°C. The cell debris was removed by centrifugation (17,500 × *g* for 30 min at 4°C), and freshly prepared 2% protamine sulfate solution was added dropwise with stirring to the clarified supernatant. After standing for 40 min, the mixture was centrifuged (17,500 × *g* for 30 min at 4°C), and the precipitate was discarded. Ammonium sulfate (65 to 90% saturation) was added to the cell extract. The resultant precipitate was collected by centrifugation and suspended in 40 mM potassium phosphate buffer (pH 7.0) followed by dialysis against the same buffer at 4°C. The dialyzed preparation was subjected to FPLC on a MonoQ HR 10/10 column and then on a Superose 12 column under the same conditions as those described for fructosyltransferase.

Assay of fructosyltransferase. The standard assay mixture contained (in 1 ml): 100 mM potassium phosphate buffer (pH 6.0), 125 mM sugar, and enzyme preparation. The reaction was run at 37°C and stopped by heating in a boiling water bath for 10 min. One unit of fructosyltransferase activity was defined as the amount of enzyme which released 1 μmol of glucose per min. Glucose (2) and fructose (1) were determined with hexokinase, glucose-6-phosphate dehydrogenase, and phosphoglucose isomerase. Reducing sugars were determined by the method of Somogyi (14). Sucrose, maltose, maltotriose, melibiose, glucosylsucrose, and maltosylsucrose were determined by liquid chromatography as described by McGinnic and Fang (13). Polysaccharide was precipitated by the addition of 2 volumes of absolute ethanol to the sample. The precipitate was collected by centrifugation (17,500 × *g* for 30 min at 4°C) and dialyzed against distilled water for 24 h. Total polysaccharide was determined by the phenol-sulfuric acid method (6), and fructan was determined by the cysteine-sulfuric acid procedure (5). When the optimum pH was studied in the pH range of 5.0 to 8.0, a buffer system was substituted with 100 mM each Tris malate buffer (pH 5.0 to 8.0), potassium phosphate buffer (pH 6.0 to 7.5), or Tris hydrochloride buffer (pH 7.0 to 8.0).

Assay of invertase. Invertase activity was measured in a standard incubation mixture containing 100 mM potassium phosphate buffer (pH 7.0), 125 mM sugar, and enzyme preparation. After incubation for 120 min at 37°C, the reaction was stopped by heating in a boiling water bath for 5 min. Glucose, fructose, and reducing sugars formed in the assay were then detected as described above.

Assay of protein. The protein concentration was measured by the method of Lowry et al. (12) with bovine serum albumin as the standard.

Electrophoresis of enzymes. Polyacrylamide gel electrophoresis of samples was done at 4°C for 2 h by the method of Davis (3) with a 7.5% gel and 5 mM Tris hydrochloride–38 mM glycine buffer (pH 8.3). After electrophoresis, the gels were removed from the tubes. To disclose the enzyme activity, the gels were incubated in 125 mM sucrose–100 mM potassium phosphate buffer (pH 6.0) at 37°C for 1 h and stained by the periodic acid-Schiff stain method described by Zacharius et al. (22). Sodium dodecyl sulfate electrophoresis

was performed by the method of Weber and Osborn (16) with 5.0% polyacrylamide gel rods. Protein bands were visualized by staining the gels with Coomassie brilliant blue.

Preparation of extracellular fructosyltransferase from *A. viscosus* NY1 and *S. mutans* NCIB 11723. *S. mutans* NCIB 11723 was grown in the medium described above at 35°C for 18 h. Cells were removed by centrifugation (8,000 × *g* for 30 min at 4°C), and the supernatant was brought to 60% saturation with ammonium sulfate and allowed to stand overnight. The precipitate containing the extracellular enzyme was collected by centrifugation and was then dialyzed for 18 h against 40 mM potassium phosphate buffer (pH 7.0) at 4°C.

A. viscosus NY1 was grown in dialyzed brain heart infusion at 35°C for 18 h. The cells were removed by centrifugation, and the culture supernatant was dialyzed for 18 h against distilled water. To absorb the enzyme, preswollen DEAE-cellulose (Whatman DE 52) was added to the dialyzed supernatant, and the suspension was stirred for 2 h at 4°C. After overnight sedimentation, the supernatant was removed, and the slurry was washed three times with 25 mM Tris hydrochloride buffer (pH 8.0). The DEAE-cellulose was then packed in a column (1.5 by 25 cm) and eluted with a gradient of 0 to 250 mM NaCl in the same buffer. The pooled enzyme fractions containing fructosyltransferase activity were dialyzed against 2 M urea containing 1% glycine.

TABLE 1. Lactate production from glucosylsucrose and maltosylsucrose by cell suspensions of streptococci, lactobacilli, and actinomyces

Strains	Relative amt of lactate produced from[a]:			
	Glucose	Glucosyl-sucrose	Maltosyl-sucrose	Turbidity at 660 nm (continuous culture)
Streptococcus sanguis				
LPH	81	30	0.4	1.40
NCTC 10904	96	0.4	0.2	1.48
ATCC 10556	140	2.9	3.2	1.38
Streptococcus salivarius				
13419	100	120	24	0.63
ATCC 9759	103	73	9.1	0.55
ATCC 8614	100	88	11	ND[b]
Streptococcus mitior				
115B2	98	70	1.4	1.18
NCTC 10712	87	11	0.0	1.02
Streptococcus milleri				
NCTC 10708	108	8.3	0.0	1.04
111C4	81	11	1.1	1.22
Lactobacillus casei IAM 1045	180	6.1	4.4	ND
Lactobacillus acidophilus IAM 1084	220	2.2	2.2	ND
Lactobacillus plantarum IAM 1041	106	6.1	0.6	ND
Lactobacillus sp. no. 3[c]	77	16	1.3	ND
Actinomyces viscosus				
NY1	88	3.8	3.8	1.24
WVU626	84	12	2.8	1.10
Frank 1	140	0.0	0.0	1.26
Actinomyces israelii WVU307	92	33	8.3	1.40
Actinomyces naeslundii NCTC 12104	21	8.8	2.9	1.00

[a] Amounts of lactate produced from the oligosaccharides (10 mM) are represented as the percentages relative to the amount of lactate produced from sucrose.
[b] ND, Not determined.
[c] This strain was isolated from human dental plaque.

TABLE 2. Production of reducing sugars released from glucosylsucrose and maltosylsucrose by cell extracts of streptococci and actinomyces

Strains	Relative amt of reducing sugar released from[a]:	
	Glucosylsucrose	Maltosylsucrose
Streptococcus sanguis		
LPH 1	1.7	1.5
NCTC 10904	4.1	4.1
Streptococcus salivarius		
13419	4.8	1.7
ATCC 9759	8.0	2.8
Streptococcus mitior		
115B2	6.2	1.1
NCTC 10712	8.3	11.0
Streptococcus milleri		
NCTC 10708	2.5	0.0
111C4	3.4	2.8
Actinomyces viscosus		
NY1	21	8.8
WVU626	63	37
Actinomyces israelii WVU307	17	12

[a] Amounts of reducing sugar released from glucosylsucrose and maltosylsucrose (100 mM) are represented as the percentages relative to the amount of lactate produced from sucrose.

Chemicals. Brain heart infusion was obtained from Difco Laboratories, Detroit, Mich. Yeast extract was from Daigo Eiyo Kagaku Inc., Osaka, Japan. L-Lactate dehydrogenase, glucose-6-phosphate dehydrogenase, hexokinase, and phosphoglucose isomerase were from Boehringer GmbH, Mannheim, Federal Republic of Germany. Glucosylsucrose [*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-β-D-fructofuranoside] and maltosylsucrose [*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-β-D-fructofuranoside] were kindly supplied by Hayashibara Biochemical Laboratories Inc., Okayama, Japan. The purity of these sugars was greater than 99%. Small amounts of contaminants in these preparations were oligosaccharides (glycosylsucrose) with a greater molecular weight than maltosylsucrose.

RESULTS

Fermentation by resting cells. Table 1 shows lactate formation from glucose, sucrose, glucosylsucrose, and maltosylsucrose by resting cells of oral microorganisms. *S. salivarius* 13419 produced comparable levels of lactate from glucosylsucrose and sucrose. This organism also metabolized maltosylsucrose more efficiently than other strains studied.

TABLE 3. Amounts of reducing sugar and fructan produced from sucrose, glucosylsucrose, maltosylsucrose, and raffinose by the decryptified cells of *S. salivarius*

S. salivarius strains	Substrate	Product[a]	
		Fructan	Reducing sugar
13419	Sucrose	118	250
	Glucosylsucrose	41	115
	Maltosylsucrose	8	60
	Raffinose	84	195
ATCC 8614	Sucrose	99	118
	Glucosylsucrose	32	40
	Maltosylsucrose	3	28
	Raffinose	93	89
ATCC 9759	Sucrose	123	263
	Glucosylsucrose	42	100
	Maltosylsucrose	5	42
	Raffinose	102	140

[a] The amount of fructan and reducing sugar from 125 mM sugar was expressed as micromoles per milliliter using fructose as a standard.

S. salivarius ATCC 9759, *S. salivarius* ATCC 8614, and *S. mitior* 115B2 produced lactate from glucosylsucrose at rates of 73, 88, and 70% of that from sucrose. Most other oral microorganisms produced only limited amounts of lactate from glucosylsucrose and maltosylsucrose. Gas chromatographic analysis revealed that no detectable amount of volatile acid was produced from glucose, sucrose, glucosylsucrose, or maltosylsucrose under these conditions. Actinomyces produced little succinate even in the presence of 10 mM $NaHCO_3$.

Hydrolysis of glycosylsucrose by cell extracts of oral microorganisms. The cell extracts of *A. viscosus* WVU626, *A. viscosus* NY1, and *A. israelii* WVU307 hydrolyzed glucosylsucrose and maltosylsucrose at a greater rate than the extracts prepared from other microorganisms (Table 2). These glycosylsucroses were, however, hydrolyzed by the cell extracts from other microorganisms to only a limited extent compared with sucrose. The reducing sugar produced from glucosylsucrose by the cell extract of *S. salivarius* 13419 was only 4.8% of that from sucrose.

Fructan production from glucosylsucrose and maltosylsucrose by decryptified cells of *S. salivarius*. The decryptified cells of *S. salivarius* 13419, ATCC 9759, and ATCC 8614 produced fructan and reducing sugars from glucosylsucrose, and the relative amounts of fructan produced were 35, 34, and 32%, respectively, of that produced from sucrose (Table 3). These cells also produced fructan and reducing sugars from maltosylsucrose. These results showed that glucosylsucrose and maltosylsucrose could serve as substrates for fructan synthesis.

TABLE 4. Purification procedure of cell-associated fructosyltransferase from *S. salivarius* 13419

Prepn	Total activity (U)[a]	Sp act (U/mg)	Yield (%)	Purification (fold)
Initial extract	4.4	0.6	100	1
Ammonium sulfate	3.0	0.8	68	1.3
Bio-Gel A-15	2.1	1.5	48	2.4
FPLC-MonoQ	0.09	15	2.0	24
FPLC-Superose 12	0.02	39	0.5	62

[a] One unit of transferase was defined as 1 μmol of glucose released per min.

Extraction of fructosyltransferase from *S. salivarius* 13419 and its purification. The cellular location of fructosyltransferase from *S. salivarius* 13419 was determined. Only 3% of the fructosyltransferase activity was contained in cell-free broth (extracellular). The cell wall fraction contained approximately 79% of the total fructosyltransferase activity of the cells. The fructosyltransferase was extracted from the cell wall and was purified as described above. The specific activity of the enzyme was increased 62-fold by the procedure described in Table 4, and the purified enzyme showed a single band on sodium dodecyl sulfate-polyacrylamide gels (Fig. 1). The molecular weight was 170,000 as determined by FPLC on a Superose 12 column. The enzyme did not show α-glucosidase activity because neither melezitose nor α-methylglucoside was hydrolyzed. No glucan was produced.

Reaction by purified fructosyltransferase. The optimum pH of the purified fructosyltransferase was 6.0. At pH 5.0 or 7.0 enzyme activity was 80% of the maximum activity observed at pH 6.0. At pHs over 8.0, the activity of the fructosyltransferase decreased rapidly with the increase in the pH. EDTA (1 mM) inhibited the enzyme activity, but the activity was restored completely by the subsequent addition of Ca^{2+}, Mg^{2+}, or Fe^{2+} (2 mM). The enzyme was inhibited by Mn^{2+} (2 mM) or Cu^{2+} (1 mM).

The reaction products formed from sucrose, raffinose, glucosylsucrose, and maltosylsucrose by the purified fructosyltransferase are presented in Table 5. The enzyme catalyzed (i) fructan synthesis from glucosylsucrose and the formation of fructose and maltose and (ii) fructan synthesis from maltosylsucrose with the formation of fructose, maltotriose, and unknown oligosaccharides. The enzyme showed a hyperbolic substrate saturation curve when sucrose or glucosylsucrose was the substrate. The V_{max} for sucrose was 44 U/h, and the K_m for sucrose was 16 mM, while the V_{max} for glucosylsucrose was 15 U/h and the K_m was 25 mM. The K_m for maltosylsucrose was 300 mM.

Purification and properties of invertase from *S. salivarius* 13419. Table 6 shows the procedure for purifying invertase from *S. salivarius* 13419. Coomassie blue staining of the final preparation showed one major protein band and one minor

FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the purified fructosyltransferase from *S. salivarius* 13419. Experimental details are in the text. A sample containing 15 μg of protein was applied.

TABLE 5. Reaction products from sucrose, glucosylsucrose, maltosylsucrose, and raffinose by fructosyltransferase of *S. salivarius* 13419[a]

Substrates	Product (μmol/ml)					
	Fructose	Glucose	Maltose	Maltotriose	Melibiose	Fructan
Sucrose	66	184	0	0	0	118
Glucosylsucrose	30	0	50	0	0	23
Maltosylsucrose	19	0	0	41	0	4
Raffinose	30	0	0	0	64	4

[a] The reaction mixture which contained (in 1 ml) 100 mM potassium phosphate buffer (pH 6.0), 125 mM sugar, and enzyme (5 μg) was incubated at 37°C for 2 h.

protein band on polyacrylamide or sodium dodecyl sulfate-polyacrylamide gels. The activity staining revealed that the major component of the protein had invertase activity. The enzyme hydrolyzed sucrose to equivalent concentrations of glucose and fructose, and neither glucan nor fructan was formed. This enzyme also hydrolyzed raffinose, giving fructose and melibiose as its products. However, little reducing sugar was released from glucosylsucrose or maltosylsucrose by this enzyme. The enzyme did not show α-glucosidase activity because it did not hydrolyze melezitose or α-methylglucoside.

There was no β-glucosidase activity because neither β-methylglucoside nor cellobiose was hydrolyzed. The enzyme activity was not dependent on P_i. The molecular weight was 68,000 as determined by FPLC on a Superose 12 column.

Reaction of glycosylsucrose with extracellular fructosyltransferase from *A. viscosus* NY1 and *S. mutans* NCIB 11723. The partially purified fructosyltransferase from *A. viscosus* NY1 produced reducing sugar and fructan from glucosylsucrose at rates of 60 and 79% of that from sucrose, respectively. It also produced fructan with a lot of reducing sugars from maltosylsucrose at the rate of 71% of that from sucrose.

The extracellular enzyme of *S. mutans* NCIB 11723 also produced fructan from glucosylsucrose and maltosylsucrose at rates of 64 and 55% of that from sucrose. This enzyme preparation produced additional polysaccharide (probably glucan) from sucrose.

No detectable amount of polysaccharide other than fructan was produced from raffinose, glucosylsucrose, and maltosylsucrose by this enzyme preparation. It was suggested that glucosylsucrose and maltosylsucrose reacted with fructosyltransferase but not with glucosyltransferase of *S. mutans* NCIB 11723.

DISCUSSION

Yamada et al. (20) have demonstrated that cell suspensions of *S. mutans* fermented glucosylsucrose or maltosylsucrose only to a small extent. In the present study, we showed that with the exception of *S. salivarius*, other predominant microorganisms in dental plaque are able to ferment these sugars to a limited extent (Table 1). *S. salivarius*, however, fermented glucosylsucrose as rapidly as sucrose, but the cell extract of *S. salivarius* did not produce more reducing sugar from glucosylsucrose than the extracts prepared from other microorganisms (Table 2). Thus, the high fermentability of glucosylsucrose by *S. salivarius* did not seem to be due to intracellular sugar-hydrolyzing invertaselike activity. Decryptified cells of *S. salivarius* produced fructan simultaneously with reducing sugar from glucosylsucrose (Table 3). This result suggested that the high fermentative efficiency of glucosylsucrose by this organism was due to the degradation of these sugars by fructosyltransferase. To confirm this possibility, we purified both invertase and fructosyltransferase from *S. salivarius* 13419 and determined their capacities to hydrolyze glycosylsucrose. Little reducing sugar was released from glucosylsucrose or maltosylsucrose after incubation with the purified invertase. In contrast, reducing sugars were released and fructan was synthesized when glucosylsucrose was incubated with the purified fructosyltransferase (Table 5). The enzyme also catalyzed fructan synthesis from maltosylsucrose. Fructose, maltotriose, and other unknown oligosaccharides were also produced. It should be noted that fructose was also released from glucosylsucrose or maltosylsucrose by this enzyme and that fructose can be easily fermented to acid by *S. salivarius*. The fructosyltransferases of *Aerobactor*, *Bacillus*, and *Actinomyces* species (7) have been reported to release fructose from sucrose in a reaction requiring water as an acceptor. Our data suggest that the fructosyltransferase of *S. salivarius* catalyzes the following reactions: (i) sucrose + $\text{fructan}_n \rightarrow \text{fructan}_{n+1}$ + glucose, and sucrose + $H_2O \rightarrow$ fructose + glucose; (ii) glucosylsucrose + $\text{fructan}_n \rightarrow \text{fructan}_{n+1}$ + maltose, and glucosylsucrose + $H_2O \rightarrow$ fructose + maltose; (iii) maltosylsucrose + $\text{fructan}_n \rightarrow \text{fructan}_{n+1}$ + maltotriose, and maltosylsucrose + $H_2O \rightarrow$ fructose + maltotriose; (iv) raffinose + $\text{fructan}_n \rightarrow \text{fructan}_{n+1}$ + melibiose, and raffinose + $H_2O \rightarrow$ fructose + melibiose.

We suggest that the cell-associated fructosyltransferase catalyzes the first step of the glucosylsucrose metabolism by *S. salivarius* and that the fructose and maltose released are then rapidly metabolized to acid. Some strains of *S. salivarius* have been known to have a fructanase (15). It is probable then that fructan is also hydrolyzed by fructanase and subsequently metabolized to acid. This may be a reason why *S. salivarius* has a high fermentative efficiency for glycosylsucrose.

In the present study, we report the presence of a cell-associated fructosyltransferase in *S. salivarius* and also extracellular fructosyltransferases in *A. viscosus* NY1 and *S.*

TABLE 6. Purification procedure of invertase from *S. salivarius* 13419

Prepn	Total activity (U)[a]	Sp act (U/mg)	Yield (%)	Purification (fold)
Crude extract	140	0.3	100	1
Ammonium sulfate	45	0.5	33	1.7
FPLC-MonoQ	12	14	9	47
FPLC-Superose 12	6	42	4	140

[a] One unit of invertase was defined at 1 μmol of glucose released per min.

mutans NCIB 11723 which can catalyze the degradation of glycosylsucrose. However, cell suspensions of these microorganisms did not produce more lactic acid than other microorganisms (Table 1), and it is possible that their extracellular fructosyltransferase was removed during the washing process. It is probable that extracellular fructosyltransferase of oral microorganisms hydrolyzes glycosylsucrose in dental plaque in situ.

The cariogenic potential of the glycosylsucrose mixture may be relatively low, because the predominant microorganisms in dental plaque have a limited low capacity to ferment glycosylsucrose. However, we should bear in mind the potential of fructosyltransferase to degrade these oligosaccharides when evaluating the cariogenic potential of these sugars.

ACKNOWLEDGMENTS

We thank John Thompson for valuable discussion and for critical reading of the manuscript.

This study was supported in part by Grants-in-Aid for Scientific Research (B) 00548306 and (A) 59440077 and Grant-in-Aid for Developmental Scientific Research (2) 60870066 from the Japanese Ministry of Education, Science and Culture.

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